arch diameter, parasternal long-axis LV area, and calculated an LV adequacy score as for AS. The broad spectrum of L hrt structure sizes did not correlate with the need for reintervention:

	Ao ann	mitral ann	LV/RV ratio	Tx arch	LV area
mean ± s.d.	6.1 ± 0.9	9.4 ± 1.6	0.06 ± 0.04	3.9 ± 0.8	4.0 ± 1.2
smallest	4.0 mm	6.0 mm	0.89	2.1 mm	1.9 cm^2

There were no deaths. Eight pts (13%) required balloon dilation for re-coarctation (reCoa). Pts having a VSD large enough to require operation were more likely to require balloon dilation of reCoa ($p < 0.02$, Fisher's Exact). At follow-up (21 ± 16 mos), 11 have AS or subAS, 10 have mild reCoa (< 20 mm Hg), and 4 have mitral stenosis. Fifty-seven pts (90%) are asymptomatic and 10% have mild symptoms. The LV adequacy score applied to these pts would have predicted survival in only 35/63 (56%). We conclude that 1) there is a broad spectrum of L hrt structure size in neonatal Coa that is not predictive of outcome, 2) a large VSD with Coa is a risk factor for reintervention, and 3) the LV adequacy score used for AS is not applicable to neonatal CoA.

815 Biology of Atherosclerosis: Nitric Oxide Synthetase, Smooth Muscle, and Plaque Stability

Monday, March 30, 1998, 2:00 p.m.–3:30 p.m.
Georgia World Congress Center, Room 257W

2:15

815-2 Endothelial Nitric Oxide Synthetase Gene Mutation Interacts With the Angiotensin Converting Enzyme Deletion Polymorphism to Increase Risk for Coronary Artery Disease

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Background: Endothelial nitric oxide synthetase (eNOS) functions in the maintenance of vascular tone and platelet aggregation and may be important in the pathogenesis of coronary artery disease (CAD). A reported G to T transition in exon 7 of the eNOS gene results in the substitution of Asp for Glu at position 298 (Asp^{298}) of the encoded protein. We tested whether Asp^{298} is associated with risk for coronary artery disease (CAD) or myocardial infarction (MI) either individually or in combination with the ACE deletion (D) polymorphism, which has been reported to increase risk for CAD/MI, possibly by interacting with Asp^{298}.

Methods: A polymerase chain reaction (PCR) amplified region of exon 7 was digested with *Sau*3AI, which detects a novel restriction site created by Asp^{298}. ACE D was determined by polymorphism analysis of PCR amplified products that include the deletion site. Products were visualized by agarose gel electrophoresis.

Results: For CAD pts (>60% stenosis, n = 261), the homozygous frequency of Asp^{298} was 7.3% vs 5.8% for controls (<10% stenosis, n = 86), p = 0.75. For MI, the frequency was 6.2% for pts (n = 113) versus 7.3% for controls (n = 234), p = 0.71. Among CAD pts, 7.6% were both homozygous for Asp^{298} and carried an ACE D allele vs 2.4% of non-CAD controls ($p < 0.05$, χ^2). Logistic regression confirmed a significant interaction between eNOS Asp^{298} and ACE D in elevating risk for CAD ($p < 0.007$) although not MI in our database.

Conclusion: eNOS Asp^{298} and ACE D interact to elevate risk of CAD. The nature of this interaction requires further study.

2:30

815-3 Smooth Muscle Cells are the Major Plasminogen-binding Site Within Atherosclerotic and Restenotic Coronary Arteries

J.J. Walter, D.C. Sane. *Bowman Gray School of Medicine, Winston-Salem, NC, USA*

Background: Plasminogen (Pg) activation within the vessel wall may play important roles in angiogenesis, smooth muscle cell (SMC) migration, and the regulation of matrix deposition. During balloon injury, plasminogen from the vessel lumen may gain access to the vessel wall, after which activation to plasmin may occur. Endothelial cells (EC) are known to have plasminogen receptors (such as annexin II) that bind plasminogen and augment its conversion to plasmin. However, whether the endothelium acts as the major site for plasminogen binding and activation is unknown.

Methods: We constructed a fusion protein consisting of the first 3 kringles of plasminogen (K3) and placental alkaline phosphatase (PAP), using the vector APtag4. The recombinant or wild type vectors were transfected into COS-1 cells, resulting in expression of PAP-K3 or PAP into serum-free medium. In situ binding studies were performed on frozen atherosclerotic or restenotic coronary artery sections. Binding was detected using BCIP/NBT.

Results: Binding of PAP-K3 (but not PAP) to adventitial microvessels, as well as to the media and neointima of larger vessels was observed. The binding was inhibitable with a 50-fold molar excess of Pg and with 10 mM EACA, confirming specific, Pg-dependent binding. The binding of PAP-K3 corresponded to areas of SMC (detected by anti-SMC actin). This binding was much more extensive than could be explained by binding to EC alone (detected by anti-CD31). [^{125}I]-Pg ligand binding assays show that the major Pg binding protein on SMC is a 52 kD band that is distinct from several known Pg or kringle-binding proteins (annexin II, cytokeratin 8, c-met).

Conclusion: The major plasminogen-binding cell within the vessel wall is the smooth muscle cell. Binding of plasminogen to SMC may regulate a variety of important vascular biological processes.

2:45

815-4 Involvement of 4-Hydroxynonenal in Vascular Smooth Muscle Cell Signaling and Atherosclerosis

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Previously we have shown the mitogenic effects of 4-Hydroxynonenal (HNE), a major lipid peroxidation product, on vascular smooth muscle cells (VSMC) in vitro. We now demonstrate by BrdU staining that HNE (2.5 μM) induced DNA synthesis in ex-vivo cultured rat aortas. In addition, HNE (2.5 and 5.0 μM) activated the transcription factor $NF_{\kappa}B$ in cultured human aortic VSMC. This effect was inhibited by antioxidants, N-acetylcysteine (NAC) and pyrrolidinedithiocarbamate (PDTC). HNE phosphorylated, but not degraded $I_{\kappa}B$-α. Treatment of VSMC with HNE induced expression of Cyclin D1 protein by 4-fold. Cyclin dependent kinase (cdk2)-activity was increased 14 h after HNE-treatment of VSMC. HNE-activated DNA-binding of the transcription factor E2F was inhibited by NAC. At higher concentrations (100 μM) HNE induced apoptosis in VSMC after 6 h of treatment, similar to the effect of TNF-α (50 ng/ml), as measured by DNA fragmentation ELISA. Aorta sections of apoE-deficient (apoE−/−) mice were analyzed by immunocytochemistry using an antibody against HNE-protein adducts. Compared to controls, the apoE−/− mice showed accumulation of HNE-adducts in the injured arteries. Plasma samples from these animals and from patients with angiographically diagnosed coronary artery disease (CAD) were analyzed for the presence of the lipid peroxidation products HNE and malondialdehyde (MDA) as well as for the oxidation product 8-isoprostane. Compared to controls, MDA + HNE levels in the apoE −/− mice and the CAD patiens were significantly elevated by 2.5-fold and 1.6-fold, respectively ($p < 0.05$, compared to controls). 8-isoprostane in the same populations was increased by 3.2-fold and 5.2-fold, respectively ($p < 0.05$). Treatment of VSMC with HNE resulted in significant elevation of 8-isoprostane after 12 h (4.5-fold, $p < 0.05$). These data further demonstrate the involvement of the lipid peroxidation product HNE in VSMC mitogenesis and atherosclerosis.

3:00

815-5 The Presence of FasL Co-localizes With Apoptosis in Human Atherosclerotic Plaques; A Possible Link in Atherosclerotic Plaque Rupture

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Background: The site of plaque rupture is characterized by a high density of macrophages and a low density of vascular smooth muscle cells (VSMC). Apoptosis of VSMC may, therefore, be an important step in weakening of the fibrous cap. We investigated the association between FasL, a cytokine that mediates cell death, and apoptosis of plaque cells in human atherosclerotic lesions.

Methods: Fourteen human coronary atherosclerotic lesions were obtained from explanted hearts from patients undergoing transplantation. Immunocytochemical analysis of these lesions was performed using antibodies against FasL (anti-FasL), macrophages (anti-CD68) and VSMC (anti-α-actin). Apoptosis was evaluated with a TUNEL assay.

Results. In 8 of the 14 coronary lesions FasL was found in the intima. In all but one lesion, expression of FasL co-localized with macrophages. TUNEL positive cells were found in 7 of the 8 FasL-positive intimas. In contrast, only 1 of the 6 FasL-negative lesions showed TUNEL positive cells ($p < 0.05$, Chi-Square). Double-staining showed that most of the TUNEL positive cells were VSMC.

Conclusion: These data suggest that the expression of FasL in human atherosclerotic plaques is associated with macrophages and apoptotic VSMC.

MONDAY ORAL